BIOSENSOR ASSAY ENHANCEMENT THROUGH AC ELECTROKINETIC MICROSTIRRING

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ABSTRACT

A microstirring tool is developed to improve binding rates in biosensor devices by an order of magnitude. AC electric fields generate fluid motion through the well documented but unexploited phenomenon, Electrothermal Flow, where the circulating flow redirects or stirs the fluid, providing more binding opportunities between suspended and wall-immobilized molecules. Simulations and experiments show improvement by a factor of 6-13. This is applicable to diffusion limited assays of different formats, from handheld microfluidic to benchtop microarray. Electrothermal microstirring is a tool that can be incorporated in existing devices, to speed up, for example, food-borne pathogen detection, or portable medical diagnostics.

1. INTRODUCTION

1.1 Immunoassays

Immunoassays, which rely on specific antigen-antibody binding for identification of proteins in a sample, have applications in both clinical laboratories for medical diagnostics and treatment monitoring, and in research laboratories for highly multiplexed testing, such as for biomarker identification. Additionally, portable immunoassays can be used for detection of environmental or food-borne pathogens, or soldier medical diagnostics. In these cases, throughput is a key consideration. One factor that can limit test duration is diffusion rate of analyte to the reporter. This is particularly true for high sensitivity ELISA tests (Stenberg; Nygren 1988). An incubation step of minutes to hours allows diffusion-limited binding to reach detectable levels.

In response to the needs for increasing throughput, portability, and sensitivity, new formats for miniaturized immunoassays have developed dramatically in recent years. These include spotted microarrays, common for "gene chips", and now used for "protein chips" (e.g. the ProtoArray from Invitrogen, Carlsbad, CA), and various forms of Lab-on-a-chip devices which can perform fluid processing and detection steps on a single chip (Northrup; Jensen et al. 2003). Small length scales permit small sample sizes and shorter test times; on-chip sample preparation reduces fluid handling steps. Though greatly aided by their small length scales, these assays can still be limited in response by diffusion of the analyte to an immobilized ligand. In this paper, we present a method where AC electrokinetics is used to enhance the performance of heterogeneous assays. Here electro-thermal forces are used to micro-stir the analyte near a functionalized surface, increasing the rate of transport to the surface. This addresses the need for faster assays by offering a tool that is adaptable to a wide variety of assay configurations and reduces the incubation time required to perform a specific test while maintaining its sensitivity.

1.2 AC Electrokinetics

AC electric fields in a microchannel or microcavity can generate forces on both the fluid itself, through AC electroosmosis and electrothermal forces, and on suspended particles through dielectrophoresis. These forces are particularly important at the micron scale where buoyancy loses its influence and where high field gradients are possible with relatively low voltages. Electrothermal flow arises from Joule heating, which produces temperature inhomogeneities and therefore temperature-dependent conductivity and permittivity inhomogeneities, which interact with the applied electric field to produce fluid motion. The electrothermal body force is given by

$$\vec{F}_{ET} = -0.5\left[\left(\frac{\nabla s}{s} - \frac{\nabla e}{e}\right)\vec{E}\frac{e\vec{E}}{1+(wt)^2} + 0.5\left|\vec{E}\right|^2 \nabla e\right] \qquad (1)$$

(Ramos; Morgan et al. 1998), where $t = e/s$ is the charge relaxation time of the fluid medium and the incremental temperature-dependent changes are

$$\nabla e = \left(\frac{\partial e}{\partial T}\right)\nabla T, \qquad \nabla s = \left(\frac{\partial s}{\partial T}\right)\nabla T. \qquad (2)$$

Electrothermal forces are particularly useful in highly conductive (*e.g.* saline buffer) solutions, where increased fluid conductivity yields more heating of the fluid at lower applied voltage.

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1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 NOV 2006	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Biosensor Assay Enhancement Through Ac Electrokinetic Microstirring	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **University of California, Santa Barbara Santa Barbara, CA 93106**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
See also ADM002075., The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**4**	

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Prescribed by ANSI Std Z39-18

2. ELECTROTHERMAL VELOCITY MEASUREMENTS

In order to measure electrothermal velocity, a planar flow device was fabricated (Fig 1). A large flow cavity was through-etched in a thin (200 μm) silicon wafer; sidewall electrodes were deposited with a gap between them (around 90 μm.) The silicon device was sandwiched between glass and filled with fluorescent tracer particles in 0.05M KCl. Micro-PIV was used to measure particle velocity and then estimate fluid velocity (Wang; Sigurdson et al. 2005). The measured velocity (Fig. 2) is on the order of 100 μm/s for 6 V_{rms} applied to the electrodes.

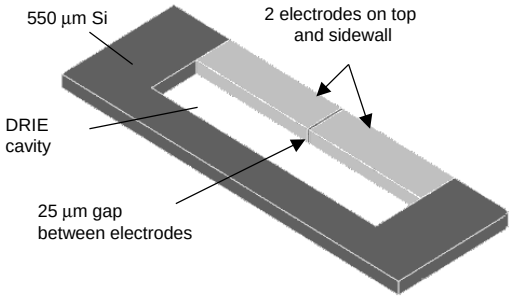

Fig. 1 Test device used to measure 2D flow created by an inhomogeneous AC electric field. The device is sandwiched between glass, and the cavity is filled with 0.05 M KCl solution seeded with fluorescent polystyrene particles. An AC driving signal produces an electric field in the fluid which generates fluid motion.

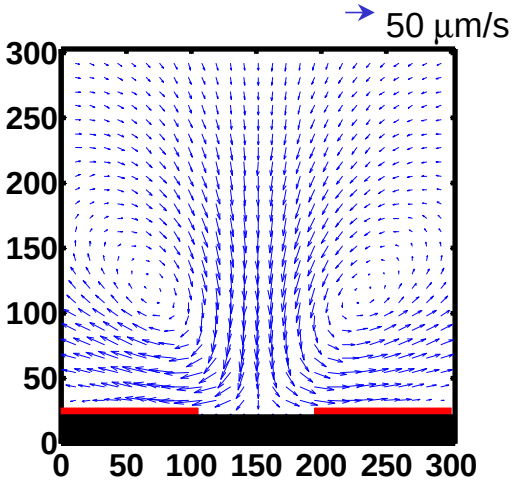

Fig. 2 Fluid velocity field measured in the device shown in Fig. 1. Images of the fluorescent particles moving in the fluid are captured at discrete times. Micro-Particle Image Velocimetry is used to determine particle velocity field. Data from two different size particle velocity fields are combined to estimate the fluid velocity. The electrodes are driven at 7 Vrms and 200 kHz. The AC field produces two counter-rotating vortices of characteristic velocity 100 μm/s. .

3. FLOW & BINDING SIMULATIONS

The finite element package *Femlab* (*Comsol*; Stockholm, Sweden) was used to solve the electric and temperature fields, and the electrothermal force (Eq. 1). The result is used as a forcing term in the Navier Stokes equations, and the velocity field is solved (Sigurdson; Wang et al. 2005). The simulation results are shown in Fig. 3, and compare well with experimentally measured velocity (Fig. 2). In order to describe how this circulating fluid motion can enhance binding rate of an immunoassay, we solved for the convection and diffusion of a scalar in the microchannel, subject to a binding boundary condition. In the passive case (Fig. 4a), a depleted cloud surrounds the binding region, and the binding rate is reduced. When electrothermal microstirring is turned on, (Fig. 4b) the depleted concentration is circulated away, and fresh analyte is circulated past the sensor. This increases binding rates.

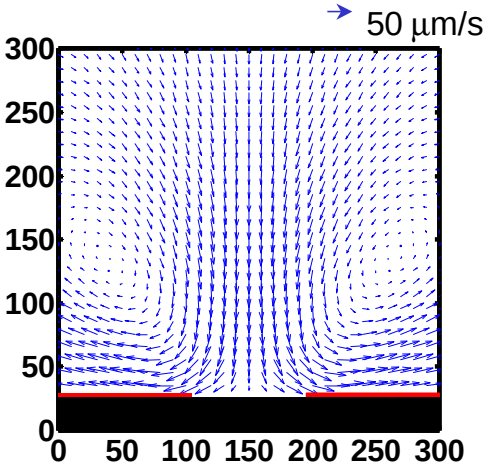

Fig. 3 Simulation of electrothermally-driven fluid motion with an applied voltage of 7 V_{rms}. The velocity of the electrothermally-driven flow is of order 100 μm/s and is characterized by a pair of counter rotating vortices. This flow closely matches the velocity field that was experimentally measured using micro-PIV (Fig. 2).

4. BINDING EXPERIMENTS

To test electrothermal microstirring with a heterogeneous streptavidin-biotin reaction, a different geometry was used. Two electrodes separated by 55 μm were deposited on silicon with an SiO_2 isolation layer (Fig 5). Because the electrothermal flow is driven by a temperature *gradient* (Eq. 2) , and the Joule heating provides a warm spot within the fluid, it is important to ensure sufficient heat removal at one or more walls in order to maintain a temperature gradient. In this device

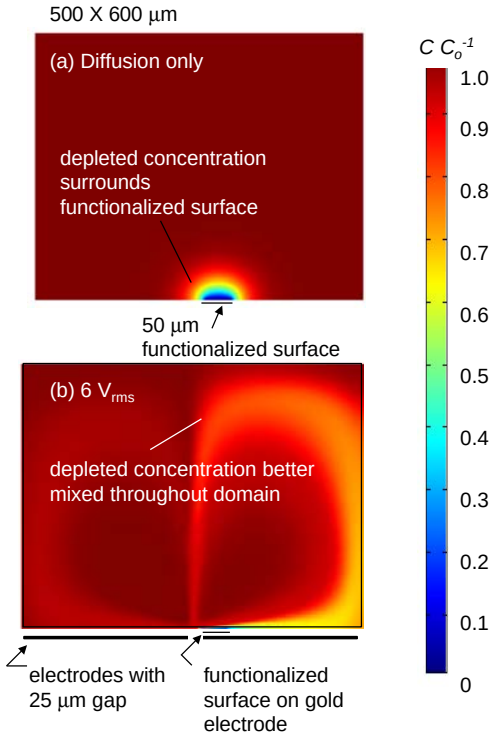

Fig. 4 Simulation of concentration in a microcavity after 100 sec, subject to binding on the functionalized surface at the wall. In (a) where mass transport is through diffusion only, a depleted cloud (blue) surrounds the functionalized surface, indicating a depletion of analyte. In (b), electrothermal flow circulates the depleted cloud throughout the cavity, and circulates fresh analyte past the functionalized surface, yielding a higher effective concentration close to the functionalized surface.

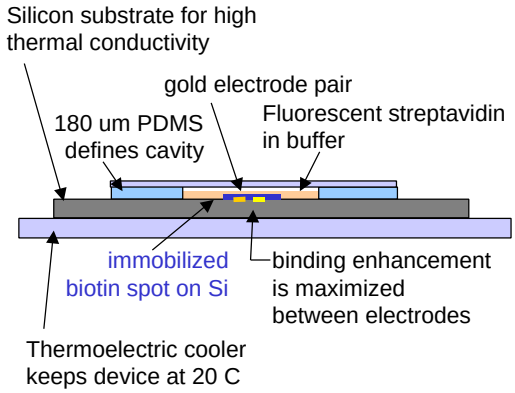

Fig. 5 Device setup for electrothermally driven microstirring enhancement of streptavidin-biotin heterogeneous assay. A 200 kHz, 10 Vrms signal is applied across the electrodes to produce electrothermal microstirring. The high thermal conductivity of the silicon base allows a sharp temperature gradient to drive the fluid, while the thermoelectric cooler prevents long-term heating of the system.

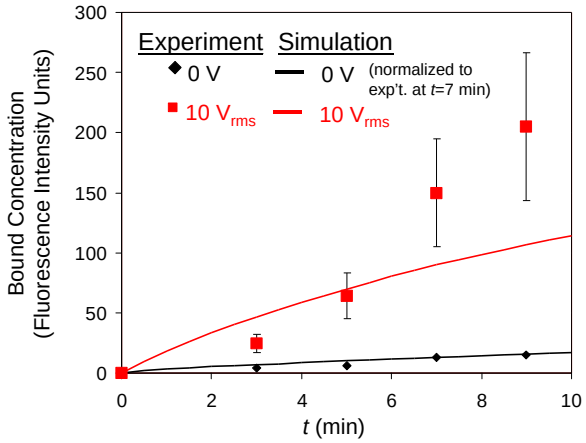

Fig. 6 Numerical and experimental binding improvement. Bound concentration with time for passive (0V or no stirring; black) and microstirred (10 V_{rms}; red). Numerical simulations (solid lines) predict a factor of 7 improvement in the microstirred case, while experimental results showmore dramatic improvement factors of 6x (at 6 minutes) to 13x (at 9 minutes). Error bars indicate experimental variation.

the silicon base is maintained at 20 C with a thermoelectric cooler. This, together with the high thermal conductivity of silicon, provides a "cold" wall, and allows a sufficient temperature gradient to drive the electrothermal flow.

A biotin spot was immobilized on the oxide layer between and around the electrodes. A 150 µm deep microcavity was defined by Parafilm walls and a glass coverslip. The cavity was filled with a fluorescent conjugate of streptavidin and the electrothermal flow was turned on. The reaction was allowed to proceed for 5 minutes, and the slide was rinsed and observed through a fluorescent microscope. The fluorescent intensity is a measure of binding. The flow-enhanced reaction is compared to the zero-flow reaction in Fig. 6, which shows a factor of 6-13 increase in bound concentration with microstirring. Fig. 6 also shows the numerical simulation predicts a factor of 7 increase in bound concentration.

CONCLUSIONS

Numerical and Experimental results point to a novel method of increasing assay speed by nearly an order of magnitude. This may be particularly useful in portable field-based medical biosensors, or environmental, or food monitoring sensors where quick results are desired. This can be useful for both the warfighter, but also for commercial applications.

ACKNOWLEDGEMENT

This work is supported by the Institute for Collaborative Biotechnologies through grant DAAD19-03-D-004 from the US Army Research Office.

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